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CONTRACTING ORGANIZATION: Nova Southeastern University

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14. ABSTRACT
The goal of the GWI consortium is to develop a better understanding of GWI and identify specific disease targets to find treatments that will address the cause of the disease. The consortium will integrate our clinical understanding of the disease process with basic research efforts using a novel mathematical model. The computational biology approach will enable the consortium to quickly identify targets of dysfunction and find treatments that will address the causes of the disease. The project will combine animal models of GWI with focus on the immune, cardiovascular and autonomic systems.

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INTRODUCTION

The underlying mechanisms of GWI remain unknown and treatment has been palliative, symptom-driven and physician-directed. The purpose of this multidisciplinary consortium project is to investigate animal GWI models with the goal of testing chemical treatments. The immune and autonomic biomarkers will be tested using a computational modeling approach allowing for a critical analysis and an accurate selection of test agents. The idea is to combine animal and human studies – a translational approach. Animal studies will be followed by clinical trials with agents thought to be most efficacious.

KEYWORDS

Autonomic Dysfunction
Computational Biology
Cytokines
Deregulated Balance
Diisopropyl Phosphorofluoridate
Electrocardiogram
Gulf War Illness
Homeostasis
Molecular Targets
Mouse Model
Putative Therapeutics
Regulatory Network Configuration
Repurposed Drugs
Sarin
Stress Response
Target Intervention
Therapeutic Interventions
Translational Human Clinical Trials
Translational Model
Treatment

ACCOMPLISHMENTS

What were the major goals of the project?

	Timeline (Months)	Percentage Complete
Major Task 1: Setup the administrative structure required for the conduct of the animal and human studies		
Subtask 1: Prepare Regulatory Documents and Research Protocols for Study 1		
Prepare, submit and receive approval for animal protocols	1-4	100%
Refine experimental protocols via conduct of preliminary experiments.	4-12	50%
Refine eligibility criteria, exclusion criteria, screening protocol	3-12	0%
Finalize consent form & human subjects protocol	3-12	0%
Submit amendments, adverse events and protocol deviations as needed	As Needed	
Coordinate with Sites for annual IRB** report for continuing review	Annually	0%
Subtask 2: Establishment of administrative structure including coordinating center and database system		
Recruit, hire and train key personnel, students, staff and faculty	1-6	90%
Setup the coordinating center including database setup	1-12	90%
Setup administrative including committee appointments and scheduling of key review meeting	1-5	100%
Development of reporting procedures – minimum of updates every 6 months.	3-12	100%
Finalize consent form & human subjects protocol , receive approval	24-36	0%
Annual meeting with the consortium members and the external advisory committee – live and via internet	As scheduled	100%
Meetings in the DC region with DoD staff and representatives of the groups – twice per year	As scheduled	100%

	Timeline (Months)	Percentage Complete
Major Task 2: Refinement and enhancement of animal models for GWI.		
Sub task 1: Establish the model of autonomic dysfunction as a surrogate for GWI.		
Train staff and students in specialized surgical methods used to setup for monitoring autonomic function.	Begin 3 and continue	50%
Test cholinergic toxins in mice with examination of peripheral autonomic and cardiac function – predict long term deficits	4-15	0%
Employ spectral analytical methods for examination of sympathetic and parasympathetic balance	Begin 4 continue	0%
Conduct wheel running acute and chronic exercise tests to simulate the exercise model in humans	4-12	0%
Combine tests of acute and chronic exercise in the GWI chemical toxin model, providing an excellent preclinical comparison.	12 continue	0%
Submit animal protocol amendments as required	As needed	100%
Measure immune biomarkers in the autonomic dysfunction model, compare to measures of adrenal function	24 continue	0%
Extend preliminary analysis to transcriptional level. Filter and normalize data using accepted best practices and perform traditional analysis of expression profiles at the level of individual genes	6-18 continue	0%
Successful use of data coordination/statistical analysis center bringing together large amounts of data from multiple systems	5 continue	0%
Subtask 2: Establish the model of DFP/cort as a surrogate for GWI.		
Train staff and students in conduct of model	Begin 3 and continue	50%
Test cholinergic toxins in mice with examination of immune markers in brain and periphery	4-15	0%
Employ analytical methods for examination of immunological balance	Begin 4 -16	10%
Establish the minimum levels of corticosterone required to maintained a heightened pro-inflammatory response to the sarin surrogate, DFP	4-12	0%
Evaluate stress regimens to establish protocols required to exacerbate proinflammatory response to sarin surrogate, DFP	5-15	0%
Submit animal protocol amendments as required	As needed	100%

	Timeline (Months)	Percentage Complete
Subtask 3: Characterize the molecular and cellular phenotypes of GWI mouse models with the idea of using them to test treatments.		
Use transcriptional analysis to study the immunological basis for the brain and blood changes in the GWI models	12 -24	2%
Use bioinformatic method to estimate pathway activation from gene expression and conduct comparisons between mouse and humans.	6-18	0%
Use molecular modeling to identify and develop networks of expression allowing for robust comparisons between GWI and animal models. Test under baseline and stimulated (stress hormones or exercise)	12-30	0%
Major Task 3: Identification of Illness specific networks with focus on human and mouse comparisons		
Subtask 1: Conduct network analysis for humans and animal models		
Apply biological modeling techniques to pathway activation computed in task 2 sub 3 to render pathway networks	6-12	0%
Integrate with other levels of biology then identify and compare functional modules at various resolutions across groups	12 -18	0%
Conduct detailed analysis of network topology applying measures of network structure and information flow to identify critical information-processing modules	12-24	0%
Conduct an analysis of the alternate steady states available to the regulatory networks identified in human and mouse models.	12-30	0%
Inform pathway-specific genomic panel based on the key network regulatory pathways	12-30	0%

	Timeline (Months)	Percentage Complete
Major Task4: Large-scale simulation of treatment.		
Subtask 1: Conduct in silico sensitivity analysis and rank candidate target nodes		
Use simulation experiments to assess and rank the impact of introducing an in silico equivalent standardized treatment pulse or pulse train at each node in turn throughout the model network	18-30	0%
Rank the candidate target nodes in terms of their relative contribution to shifting the structure of the network recovered under treatment and the network presented in healthy control subjects	18-30	0%
Major Task 5: Define and deploy large-scale optimization.		
Subtask 1: Evaluate and select the best global search algorithm for targeting intervention possibilities		
Review latest developments in evolutionary programming techniques as well as hybrid gradient-based techniques to determine the most suitable search algorithm. Acquire or develop code and deploy.	12-18	0%
Configure simulation-based optimization scheme that evaluates the fitness of candidate interventions by repeatedly launching short network simulation runs in search of the most robust treatment course	18-24	0%
Major Task 6: Identify candidate treatment courses for GWI		
Subtask 1: Using task 5 launch optimization runs from multiple initial conditions of endocrine-immune status		
Identify and describe mathematically the immune and endocrine descriptors that can be effectively and safely changed and over what range they may be changed.	24-30	0%
Using drug databases and bioinformatic techniques identify drugs currently available for repurposing to treat GWI	12-30	0%
Search for novel treatment courses. Launch repeated searches for optimal treatments using the set of candidate cytokine, hormone/autonomic and immune markers isolated in task 5	24-36	0%

	Timeline (Months)	Percentage Complete
Major Task 7: Identify candidate treatment courses for GWI		
Subtask 1: Select and test pharmacological therapies on basis of data from computational models in animals		
Use previous data to select best animal models based on immunological and autonomic biomarkers	24-36	0%
Develop computer/mathematical paradigms for evaluation of treatment strategies	12-30	0%
Develop pilot clinical trials on basis of animal studies	24-36	0%
Major Task8: Verify treatment effectiveness in human subjects		
Subtask 1: Studies of treatment effectiveness in humans		
Design assessment platform for use in human translational studies using the RedCAP platform as a foundation	18-24	0%
Complete the IRB process for selected study drugs, using the Miami VAMC IRB with OCMR review.	24-30	0%
Recruit and perform assessments of GWI subjects on intervention(s) in the phase 1 translational studies.	30-40	0%
Evaluate change in network interactions from interventions suggested Study 3 and 4. Inform the model with the human study data and refine as necessary	32-48	0%

What was accomplished under these goals?

- The IACUC and ACURO approvals have been obtained for the animal use protocols (**Task 1; Subtask 1**).
- Most of the personnel have been hired and trained (**Task 1; Subtask2**). The remaining two positions will be hired and trained shortly.
- Conducted and attended a workshop in the application of ECHO and ultrasound (**Task 1; Subtask2)**
- We have set up an Executive Board that meets regularly to discuss and review the project (**Task 1; Subtask 2**).
- We have met twice with the DoD staff and representatives (**Task 1; Subtask 2**). The first was for the Post Award Kick-Off Meeting on February 26, 2014 in Gaithersburg, MD. The second was for the EAB meeting on September 15, 2014 at Ft. Detrick.
- Implemented prototype Oracle research database (**Task 1; Subtask 2**). Now have regularly scheduled upload of clinical data from the RedCap database as well as all laboratory assay results into a central Oracle database with full query, archive and security functions (**Figure 1, See Appendices**).
- Conducted analysis of association patterns linking markers of autonomic during exercise in male and female mice, both trained and sedentary (**Task 2; Subtask 1**).

These pointed to markedly different patterns across the sexes. For example a stronger and more tightly coupled negative association between pulse interval and arterial pressure was found in males versus females.

- Conducted analysis of differences in symptom profiles and patterns of immune marker co-expression in clusters of GWI subjects defined on the basis of Davidson Trauma score profiles (**Task 2; Subtask 1**). Clusters differed significantly in exercise capacity and presented with characteristic differences in cytokine expression. We are currently increasing the statistical power of this analysis. Early results suggest that mouse exposure models should be evaluated against trauma-delimited sub-groups of GWI subjects instead of a single mixed population.
- Conducted analysis of cytokine profiles in mouse brain and blood emerging in response to handling stress (**Task 2; Subtask 2**). Identified emergent networks centered about IL-4 and IL-12 in brain.
- Conducted trial network analysis of stress-immune potentiation in mice (**Task 2; Subtask 3**). Performed trail analysis of association network properties linking blood-borne cytokine concentrations and expression of immune messenger RNA in hippocampus and cortex in mice. Robust network structures were found between markers in these compartments for mice challenged immunologically with LPS following prior chronic exposure to corticosterone. No such associations emerged in mice challenged only with LPS or corticosterone. A manuscript has been prepared and is now under internal review (**Figure 2, See Appendices**).
- Explored possible PTSD co-morbidity effects (**Task 3**). Conducted preliminary pilot analysis immune marker profile differences in sub-groups of GWI subjects clustered on the basis of the three constituent scales of the Davidson Trauma Score (DTS). Clustering in a group of n=21 male GWI subjects recruited and assessed under Dr. Klimas's prior award (W81XWH-09-2-0071) revealed a group of n=15 high trauma and n=6 low trauma subjects. A first comparative statistical analysis revealed significant differences between trauma sub-groups in exercise capacity, fatigue, social function and IL-4 expression (**Figure 3, See Appendices**).
- Conducted hypothesis driven analysis of transcription factor and target gene expression in pathways thought to be involved in cognition and fatigue (**Task 2, Subtask 3**). Confirmed differential expression of genes involved in data collected in human subjects under Dr. Klimas's prior award (W81XWH-09-2-0071). In addition to significant differences in the expression of transcription factor c-JUN in GWI, 6 of the 10 pathways found to involve c-JUN in the KEGG database contained other genes significantly expressed in GWI. These pathways supported immune and endocrine signaling such as TNF signaling (LTA, TNF, MAP3K7IP) and GnRH signaling (GNAS). This material is being assembled for an upcoming poster presentation.
- Developed custom file parser to extract and make available heart rate data from exercise recorder (**Task 2, Subtask 1**).

<u>Progress at CDC site</u>:

- Staffing is underway; offer made to postdoctoral fellow candidate and accepted.
- All ACUROs approved and shipping arrangements between CDC and Miami VA are approved.
- CDC finally received funds and spending authority in late June.

- CDC has validated their key approaches: response of microglial and astroglial transgenic mice to neurotoxicants.
- Validated brain vs. serum cytokine expression measures.
- Mice are on order or bred for start of dosing in FY 15 (Oct. 1).

What opportunities for training and professional development has the project provided?

- We recruited 2 graduate students and 1 undergraduate summer student into research internships from May to August 2014. These students conducted exploratory analyses directed at exploring improved stratification strategies for GWI cohorts and validation of gene expression in pathways thought to affect cognition.
- Trained 2 research assistants in the use of the echocardiogram machine and the mouse protocols.

How were the results disseminated to communities of interest?

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

- We anticipate that our goal oriented progress will be greater in year 2. The personnel have been hired and trained and the protocols are active.
- Will begin IRB and DoD approvals for human subjects in year 2.

IMPACT

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

CHANGES/PROBLEMS

Changes in approach and reasons for change
The approach is the same as delineated in the consortium document

Actual or anticipated problems or delays and actions or plans to resolve them
- CDC finally received funds and spending authority in late June
- Delay is getting animal protocol approval and Miami VA, which also delayed ACURO approval

Changes that had a significant impact on expenditures
Due to delays, expenditures are significantly less than what they should be. We are on an accelerated timeline and should be back on track with expenditures and reserach by the end of year 2.

Significant changes in use or care of human subjects
Nothing to Report

Significant changes in use or care of vertebrate animals
Nothing to Report

Significant changes in use of biohazards and/or select agents
Nothing to Report

PRODUCTS

Publications, conference papers, and presentations

Journal publications.
Nothing to Report

Books or other non-periodical, one-time publications.
Nothing to Report

Other Publications, conference papers, and presentations.
Nothing to Report

Website(s) or other Internet site(s)
Nothing to Report

Technologies or techniques
Nothing to Report

Inventions, patent applications, and/or licenses
Nothing to Report

Other Products
Nothing to Report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Mariana Morris, PhD
Project Role:	PI
Research Identifier:	eCommons: mariana
Nearest person month worked:	3
Contribution to Project:	Overseeing the entire research project. Established the animal protocols and in charge of the animal research. Oversees hiring of all personnel.
Funding Support:	NIH

Name:	Gordon Broderick, PhD
Project Role:	Co-Director
Research Identifier:	eCommons: gbroderick
Nearest person month worked:	3
Contribution to Project:	Head of computational biology. Has worked on the computational models for animal and human research to assist in protocols and findings.
Funding Support:	NIH, VA

Name:	Travis Craddock, PhD
Project Role:	Co-Investigator
Research Identifier:	eCommons: TRAVISCRADDOCK
Nearest person month worked:	3
Contribution to Project:	Has worked on the computational models for animal and human research to assist in protocols and findings.
Funding Support:	NIH, CFIDS Association of America, Nova Southeastern University PFRDG

Name:	Nancy Klimas, MD
Project Role:	Co-Director
Research Identifier:	eCommons: nklimas
Nearest person month worked:	1
Contribution to Project:	Head of clinical sciences. Reviewed modeling from the computational biology team in regards to human subjects to help establish protocols.
Funding Support:	NIH, VA, CDC

Name:	Mary Ann Fletcher, PhD
Project Role:	Co-Investigator
Research Identifier:	eCommons: mfletche
Nearest person month worked:	2
Contribution to Project:	Director of the immunology core.
Funding Support:	NIH, VA

Name:	James Blount
Project Role:	Administrative Coordinator
Research Identifier:	None
Nearest person month worked:	7
Contribution to Project:	Monitored budget, maintained meeting schedules, prepared quarterly and annual reports, assisted in establishing sub-awards, and other duties associated with administration of the award.
Funding Support	None

Name:	Ana Del Alamo
Project Role:	Research Associate
Research Identifier:	None
Nearest person month worked:	4
Contribution to Project:	Assisted Dr. Klimas in in her work concerning the human subject protocols.
Funding Support:	None

Name:	Mark Rice
Project Role:	Data Control Specialist
Research Identifier:	None
Nearest person month worked:	12
Contribution to Project:	In charge of the data analysis and has assisted on the computational models for animal and human research to assist in protocols and findings.
Funding Support:	None

Name:	Jacqueline Machi
Project Role:	Visiting Scholar/Graduate Student
Research Identifier:	None
Nearest person month worked:	6
Contribution to Project:	Active in animal experiments
Funding Support:	NIH

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
No

What other organizations were involved as partners?

Name:	Centers for Disease Control and Prevention National Institute for Occupational Safety and Health
Location:	1095 Willowdale Road Morgantown, WV 26505
Contribution:	Chemical toxicology project collaboration
Financial:	None
In-kind Support:	None
Facilities:	None
Collaboration:	Partner's staff works with project staff in the project.
Personnel Exchanges:	None
Other:	None

Name:	Southwest Research Institute
Location:	5220 Culebra Road, PO Drawer 28510 San Antonio, TX 78228
Contribution:	Assisting on drug choices to test in animals and humans.
Financial:	None
In-kind Support:	None
Facilities:	None
Collaboration:	Partner's staff works with project staff in the project.
Personnel Exchanges:	None
Other:	None

Name:	South Florida VA Foundation for Research & Education Inc.
Location:	1201 NW 16th Street, Room #2A103 Miami, FL 33125
Contribution:	Providing subjects and space for human trials in future. Help with establishing human protocols.
Financial:	None
In-kind Support:	None
Facilities:	Project staff uses the partner's facilities for project activities.
Collaboration:	Partner's staff works with project staff in the project.
Personnel Exchanges:	Project staff uses each other's facilities. Dr. Klimas and Dr. Fletcher are on staff at Nova Southeastern University and the Miami VA.
Other:	None

Name:	South Florida VA Foundation for Research & Education Inc. – Animal Facility
Location:	1201 NW 16th Street, Room #2A102 Miami, FL 33125
Contribution:	
Financial:	None
In-kind Support:	None
Facilities:	Project staff uses the partner's facilities for project activities.
Collaboration:	Partner's staff works with project staff in the project.
Personnel Exchanges:	Project staff uses each other's facilities. Dr. Morris is on staff at Nova Southeastern University and the Miami VA.
Other:	None

SPECIAL REPORTING REQUIREMENTS

Collaborative Awards:
Nothing to Report

Quad Charts:

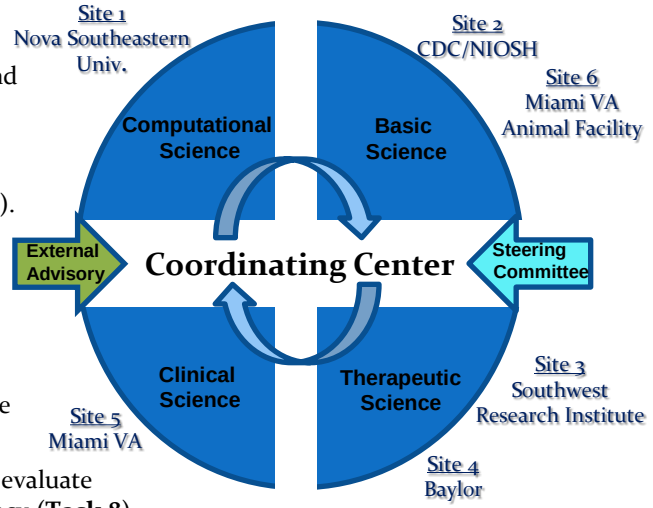
Understanding Gulf War Illness: An Integrative Modeling Approach

Award Number: GW120045 / W81XWH-13-2-0085

PI: Dr. Mariana Morris **Org:** Nova Southeastern University **Award Amount:** $4,102,527

Approach To develop a translational model of GWI for rapid identification of molecular targets and prediction of effective therapeutic interventions. The effectiveness of candidate treatment in terms of system abatement and recovery of regulatory network configuration will be assessed in GWI subjects in phase 1 translational studies.

- ❑ ***Study 1:*** Characterize the autonomic neural/adrenal dysfunction in a mouse model of GWI using validation and direction from computational biology (**Task 2**).
- ❑ ***Study 2:*** Characterize the molecular and cellular phenotype of GWI in a mouse model to evaluate the role of stress response in persistence of the illness (**Task 2**).
- ❑ ***Study 3:*** Integrate human (previously completed) and animal studies using computational biology to identify mediators of deregulated balance and test putative therapeutics (**Task 3-5**)
- ❑ ***Study 4:*** Evaluate therapeutics suggested by computational model in GWI animal models. Two or three most favorable will move on to human testing (**Task 6-7**).
- ❑ ***Study 5:*** Perform translational human clinical trials to evaluate homeostasis "reset" as well as preliminary safety and efficacy (**Task 8**).



Accomplishments to date

1- CDC IACUC approval obtained and protocol has been submitted to DoD ACURO

2- Miami VA animal protocol approved by VA IACUC. Sent to DoD ACURO and awaiting DoD approval

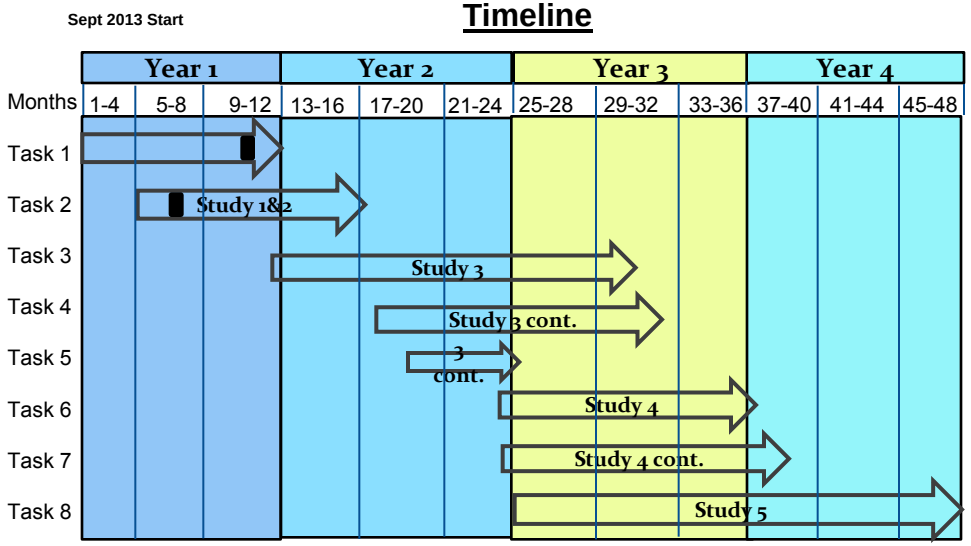
3- Administrative structure of the coordinating center (new hires) and database system have been established

4- Autonomic dysfunction studies have been initiated. Results to date:

a) tightly coupled negative association between pulse interval and arterial pressure in males vs female mice during exercise

b) emergent networks centered on IL-4 and IL-12 in brain following analysis of cytokine profiles in mouse in response to handling stress - manuscript currently in preparation

Timeline



Goals/Milestones

FY13 Goal – Administrative structure for animal/human studies (Task 1)

- ☑ Kick-off meetings with GWIRP staff and study PIs
- ☑ Protocol preparation and initiation of approvals for animal/human use
- ☑ Coordinating center database set-up

FY14 Goal – Studies 1- 3 - Refinement and enhancement of models for GWI

- ☐ Establish model of autonomic dysfunction as a surrogate for GWI (**Task 2**)
- ☐ Identification of Illness specific networks with focus on human and mouse comparisons (**Task 3**)
- ☐ Large-scale simulation of treatment. (**Task 4**)
- ☐ Define/deploy optimization and target intervention possibilities (**Task 5**)

FY15 Goal – Study 4 - Candidate treatment courses

- ☐ Identify candidate treatment courses for GWI (**Task 6**)
- ☐ Select and test therapies in animals (**Task 7**)

FY16 Goal – Study 5 - Perform translational human clinical trials

- ☐ Verify treatment effectiveness in human subjects n=30 (**Task 8**)

APPENDICES

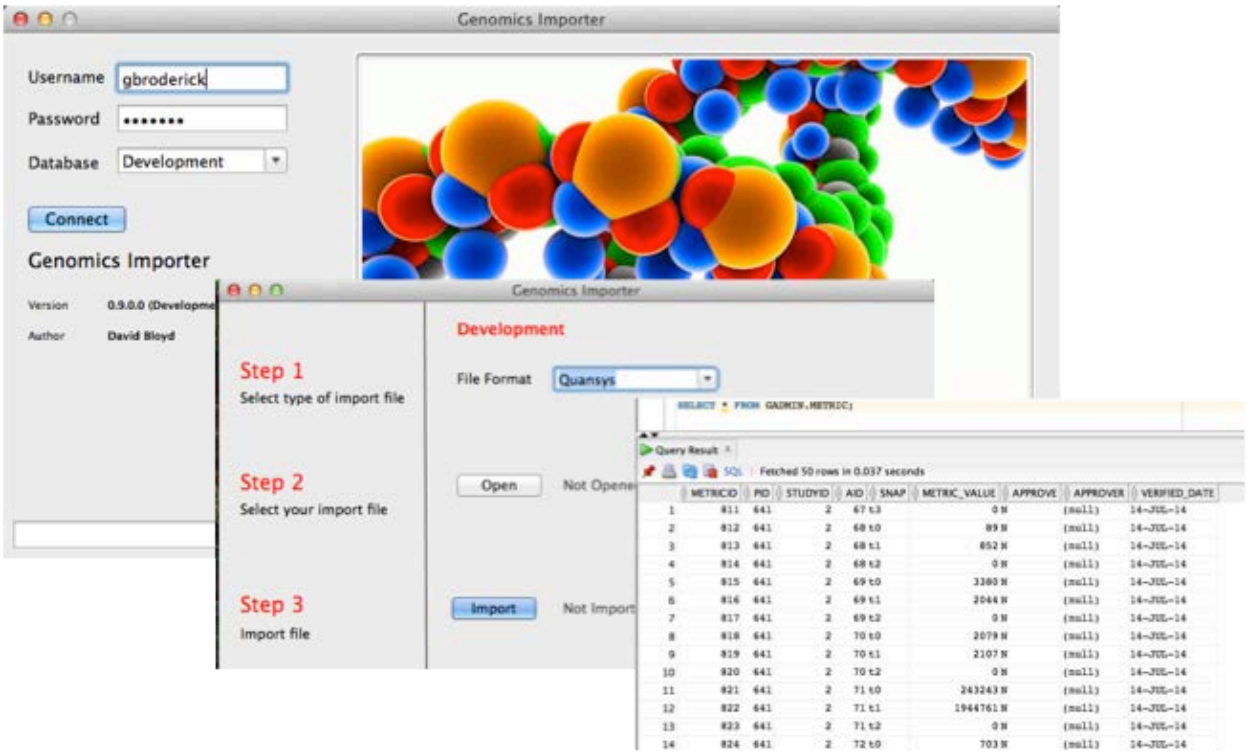


Figure 1. Basic data query interface for Oracle Consortium research database.

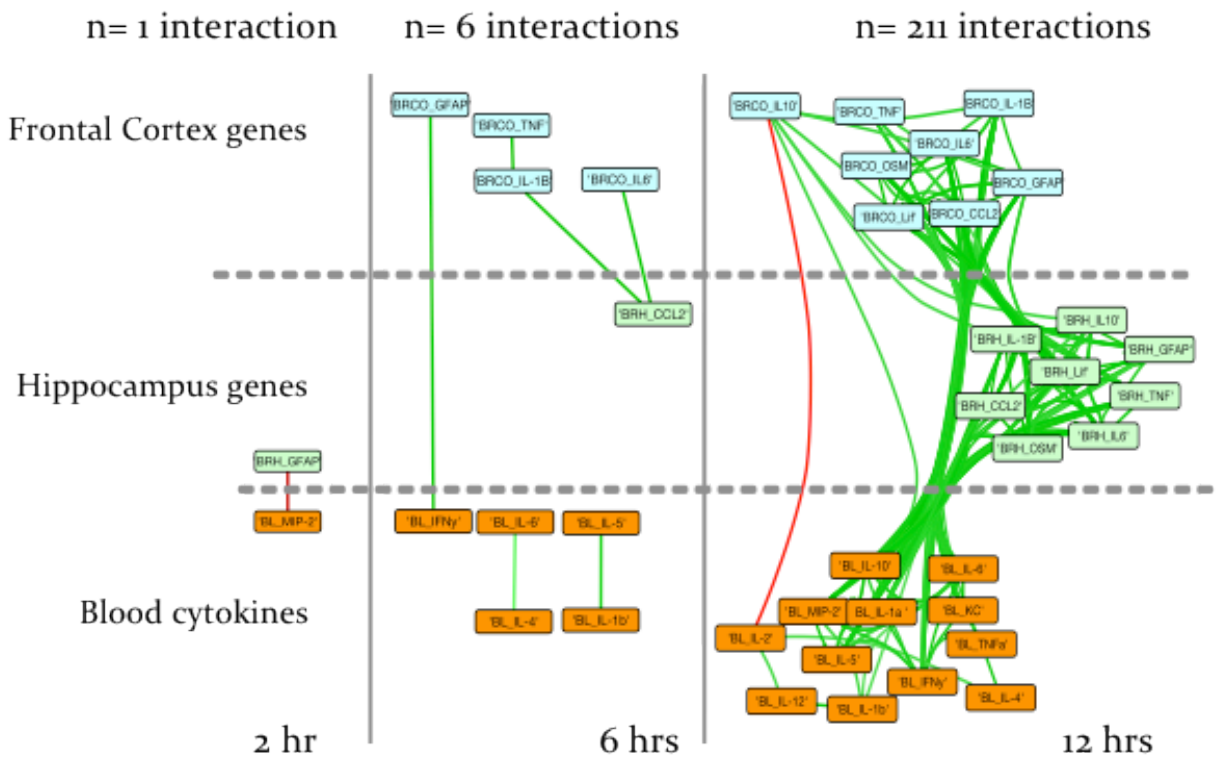


Figure 2. Association networks based on Pearson correlation constructed in mice at 2, 6 and 12 hours following immune challenge with LPS when mice were first subjected to sustain treatment with corticosterone. Line thickness is proportional to strength of correlation, green indicating positive correlation and red negative correlation.

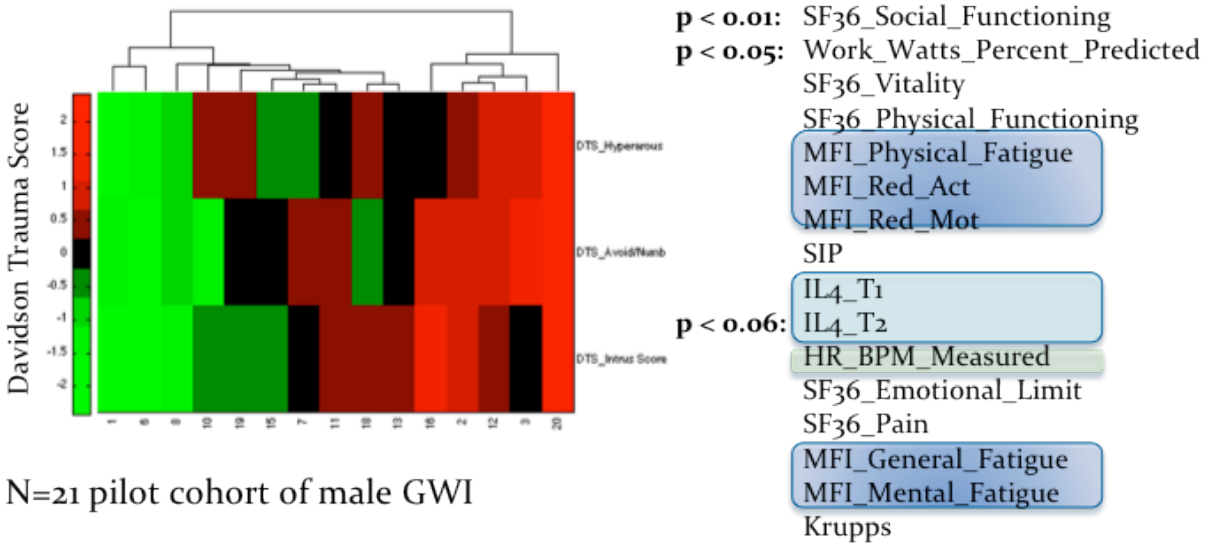


Figure 3. Preliminary stratification of male GWI subjects according to Davidson Trauma profile suggests significant differences may exist in high-trauma versus low-trauma subgroups, in terms of fatigue, social function, exercise capacity and IL-4 expression. This remains a small group size and further analysis is required.